10/534801

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Description of the method

First step

Small pieces of plant leaves (5 mm in diameter) are placed in a microtiter plate (with flat bottom holes) filled with 50 µl of a cocktail containing different carbohydrases in a DNA preserving medium.

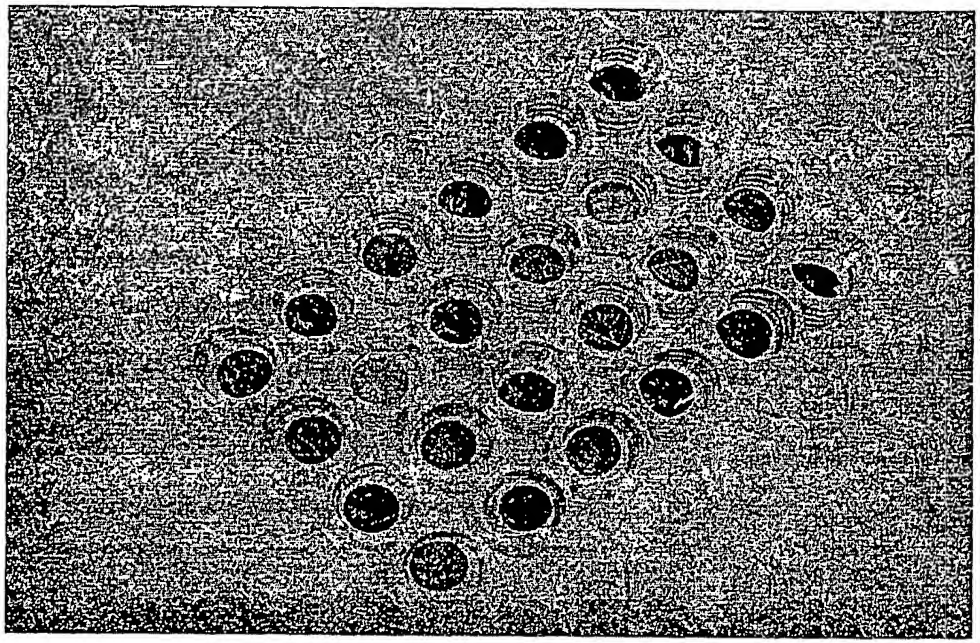
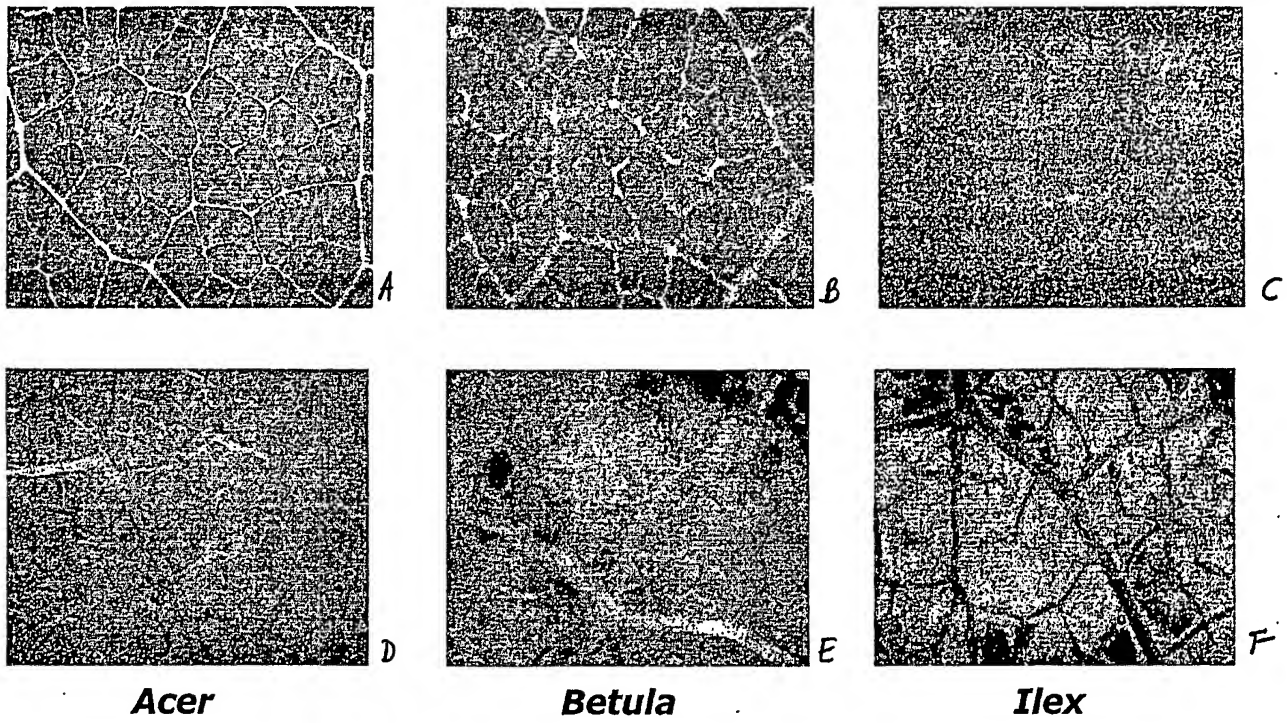


FIGURE 1

Microtiter plates are closed with sellotape and incubated overnight at 50°C with a strong agitation. Because of the cell wall digestion, the leaves desintegrate with their cells separated from each other.



Microscopic aspect of leaves of Acer, Betula *and* Ilex *before and after digestion*

FIGURE 2

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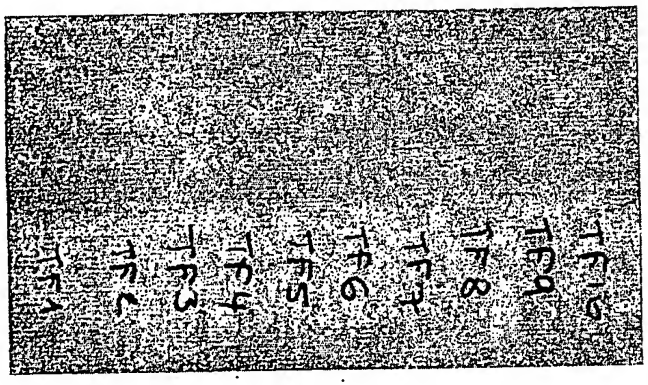
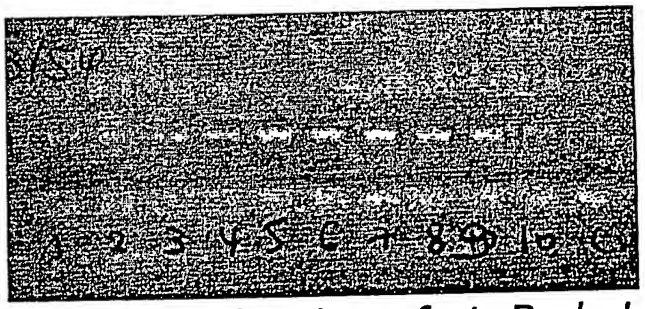
PCR results from DNA extracted with the enzymatic method on silica gel-dried leaves of 10 specimens of *Ilex perado* collected in the Canary islands one year ago.

A

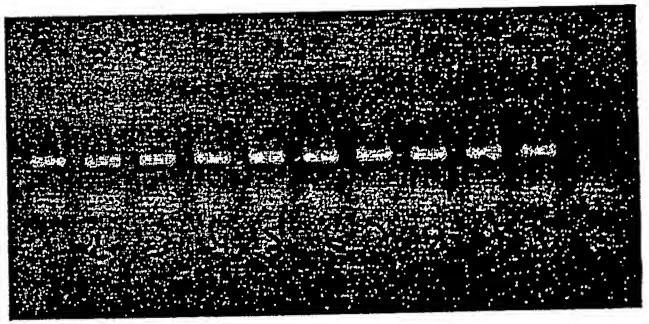


Isolated genomic DNA (1)

B



PCR amplification of atpB-rbcL (plastid DNA)



PCR amplification of ITS (nuclear DNA)

C

(1) The sellotape of the first (TF1) and last (TF10) samples leaked during the overnight incubation

FIGURE 3

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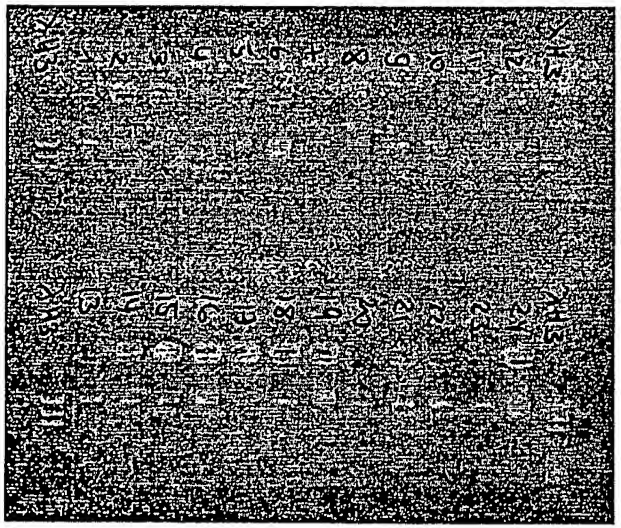
Second step

After overnight incubation, 30 µl of the cell homogenate are treated as starting material for DNA isolation using current and standardized protocols compatible with automation. Magnetic beads can be used (for instance those proposed by DYNAL, AGOWA, QIAGEN, BILATEC, ROCHE, PROMEGA, GENOVISION ...etc.).



Ethydium bromide agarose gel of different DNA isolated with the enzymatic method for 24 different species. The name of these species are indicated in the list of species successfully extracted until now. 25% of the extracted DNA is loaded in the gel.

FIGURE 4

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